Reactivity to Stress in Rats as a Function of Infantile Experience.* (30292)

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Levine(4) has suggested that rats stimulated during infancy may be more reactive to, but also recover more rapidly from, stress as adults than non-stimulated rats. These rats have a higher output of corticosterone and steroid production is greater in the 15-minute period following stress. Similarly, Bell, Reisner, and Linn(1) reported that recovery from electroconvulsive shock (ECS) is more rapid in rats handled the first 5 days of life, as compared with rats that were either handled or unhandled at later periods. They defined recovery from ECS by blood glucose levels taken 24 hours after ECS and immediately following ether anesthesia. Blood glucose levels did not differ for subjects handled the first five postnatal days that did or did not receive ECS. Furthermore, differences were found for subjects not so handled. Since ether anesthesia is a stressor in and of itself, however, 2 alternative conclusions could be drawn. Recovery from ECS may have been complete in all subjects 24 hours after ECS. Consequently, the difference between the rats handled the first 5 days of life and the other groups showed these rats less reactive to the immediate stress of ether anesthesia. Or, conversely, possibly none of the rats subjected to ECS had recovered within 24 hours. The rats handled the first 5 days of life may have been more reactive to the etherization.

To test Levine's hypothesis, but unconfounded with ether anesthesia, the following experiment was conducted. One hundred and ninety-one Sprague-Dawley rats, 99 females and 92 males, were either handled or unhandled from days 2 through 5 of life, and either stressed or not stressed in adulthood. The handling procedure was removal of the litter from the mother. Each pup was then placed in a small glass dish for 3 minutes before being returned to the mother. For purposes of another experiment, from days 7 through 21, each rat was also given one 3-minute test for activity level in a 15½ × 15½ × 6-inch plexiglass box in near darkness. However, assignment to groups in adulthood was such that the effects from this testing were equally distributed throughout all groups.

The subjects were weaned and weighed at 21 days, housed in groups of 3 to 4 littermates, and left undisturbed until between 55 and 60 days of age. At 9:00 p.m. the night before the subjects were to be stressed and guillotined, food was removed from their cages. Thirteen hours later the rats to be stressed were given either a one-minute ice water bath, dried, and placed individually in a new cage, or they were only moved to a new cage. Non-stressed controls were left alone. Either 1, 30, 60, or 80 minutes following stress, the subjects were guillotined, and blood samples taken and analyzed for glucose levels by a modification of a method proposed by Hoffman(3).

No significant differences in 21-day body weights as a function of Sex or Infantile Experience were found. Analysis of variance indicated that all main effects were significant. There were significant differences in glucose levels attributable to Type of Stress ($p<.001$), Time Since Stress ($p<.05$), Infantile Experience ($p<.001$), and Sex ($p<.025$). In addition, the Stress × Time Since Stress interaction was significant ($p<.001$), and the Infantile Experience × Sex interaction approached significance ($p<.06$). Mean glucose levels in milligrams per cent as a function of Stress, Infantile Experience, and Sex, are shown in Fig. 1. Specific comparisons showed that non-stressed rats had lower blood sugar levels than the two groups of stressed

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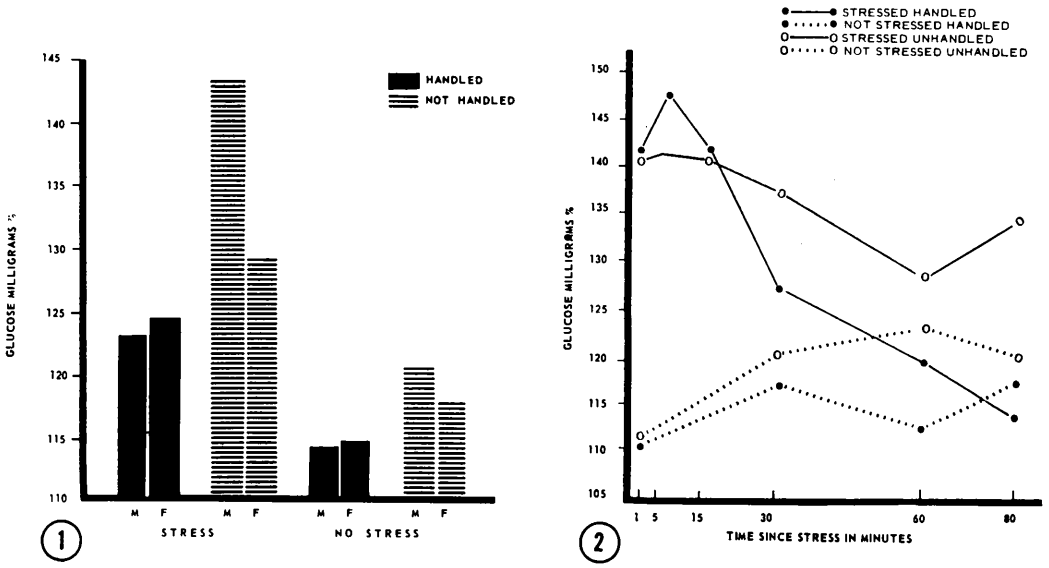

FIG. 1. Mean glucose levels in milligrams % as a function of stress, infantile experience, and sex.

FIG. 2. Mean glucose levels over time as a function of stress and infantile experience.

rats ($p<.001$), which did not differ from one another. The Time Since Stress effect was shown in the drop in glucose levels over time. One minute after stress, glucose level averaged 128.1. This had dropped to 123.5 after 80 minutes. Unhandled rats had higher glucose levels than handled rats, and males had higher blood sugar levels than females. The source of the Stress $\times$ Time Since Stress interaction was the more rapid recovery of ice water stressed subjects compared to the new cage subjects. Specific comparisons indicated that the 2 stressed groups had significantly higher glucose concentrations than the controls 30 minutes after stress ($p<.05$). However, by the time 80 minutes had elapsed, the ice water and control groups did not differ, but both averaged lower than the new cage subjects ($p<.01$). While the Infantile Experience $\times$ Sex interaction did not quite reach significance, the data suggest that the handling effect may be attributable to males rather than females.

In order to assess whether stimulated and non-stimulated rats differ in initial reactivity to stress, another 72 subjects, 37 males and 35 females, were stressed either by an ice water bath or confinement to a new cage, and sacrificed either 5 or 15 minutes following stress. The data were then reanalyzed at 6 time points, with the dimensions consisting of Infantile Experience, Sex, and 2 Stress conditions. The results were the same as indicated by the first analysis with two exceptions. There was no Type of Stress effect, but the Time Since Stress $\times$ Infantile Experience interaction was significant ($p<.025$). Specific comparisons indicated that handled rats had significantly lower blood sugar levels 80 minutes after stress than unhandled rats ($p<.001$).

Responses to stress *vs* non-stress over time as a function of infantile experience are summarized in Fig. 2. From these data, no conclusions can be drawn with regard to the initial portion of Levine's hypothesis, namely, that handled subjects are more reactive initially. A specific comparison between handled and unhandled rats 5 minutes following stress indicated that the difference was insignificant, even though it was in the appropriate direction. However, it is clear that stressed handled rats recover more quickly than stressed unhandled rats. Specific comparisons made between stressed handled subjects and the control subjects 30 minutes after stress indicated stressed handled subjects did not differ from either control group. Stressed

unhandled rats did differ significantly from the controls, however, ($p<.025$). Sixty minutes after stress, the only groups which differed from one another were stressed unhandled rats and non-stressed handled rats. However, 80 minutes after stress, stressed unhandled subjects differed significantly ($p<.025$) from all other groups, which did not differ from one another. Therefore, the hypothesis that rats stimulated in infancy recover from stress more rapidly than non-stimulated rats is supported.

The other results of particular interest are the significant Sex difference, and the Sex $\times$ Infantile Experience interaction. The higher glucose levels found in males, as compared with females are consistent with the sex differences found with other measures frequently assumed to reflect emotional reactivity(2). Differential reactions of the sexes to infantile stimulation are not usually reported. Levine(4), however, found that stimulated rats survived leukemia longer than non-stimulated rats. This effect was due to the difference between stimulated and non-stimulated males. The results of the present experiment, which suggest that the handling effect may be attributable to males rather than females, are consistent with these findings.

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Radiosensitivity and Biological Properties of Two Tumor Types Indigenous to the Same Host. IX. Swelling Properties of Mitochondria Isolated from Mouse Tumor and Liver Tissue.* (30293)

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During investigation of the biological properties of tumor types, we investigated the swelling phenomenon of their mitochondria under various conditions of isolation, resuspension in various media, and incubation. Previous electron microscopic studies revealed a difference in morphological structure of mitochondria of the 2 tumor types(1). Differences in oxidative phosphorylation of the 2 tumor types were also ascertained(2), and some enzymic and metabolic differences between neoplastic and normal tissue mitochondria have been discussed by Kit and Griffin (3).

Mitochondrial swelling can be induced by various agents such as phosphate and thyroxin. The action of these agents is thought to be mediated by their effect upon the electron transport system of this organelle(4,5). Other compounds, such as ascorbate(6) and glutathione(7,8), are believed to exert their effect directly upon the mitochondrial membrane. Spontaneous swelling of mitochondrial preparations has also been reported(9,10).

Evidence is herein presented showing dependence of the rate and extent of swelling on the quality of the isolated mitochondrial fraction of 2 mouse tumor types and of mouse liver.

Materials and methods. Two tumor types, an epithelial cell, slow growing, and a spindle cell, fast growing, served as basic test material. Both tumors had originated in the mammary glands of female mice of the inbred DBA/212 strain and are being carried in isologous parent hosts by serial transplants. Liver mitochondria from normal mice of the same strain were assayed as controls for comparative purposes.

The isolation of mitochondrial fractions

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